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Seasonal changes in root and soil respiration of ozone-exposed ponderosa pine (*Pinus ponderosa*) grown in different substrates

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SUMMARY

Exposure to ozone (O_3) has been shown to decrease the allocation of carbon to tree roots. Decreased allocation of carbon to roots might disrupt root metabolism and rhizosphere organisms. The effects of soil type and shoot O_3 exposure on below-ground respiration and soil microbial populations were investigated using container-grown ponderosa pine (*Pinus ponderosa* Laws.) growing in a low-nutrient soil, or a fertilizer-amended organic potting media, and exposed to one of three levels of O_3 for two growing seasons in open-top exposure chambers. A closed system, designed to measure below-ground respiratory activity (CO_2 production, O_2 consumption and RQ-Respiration Quotient; (CO_2:O_2) of plants growing in pots, was used monthly to monitor below-ground respiration of 3-yr-old ponderosa pine.

Although seasonal differences were detected, CO_2 production (μmol h^{-1} g^{-1} total root d. wt), O_2 consumption (μmol h^{-1} g^{-1} total root d. wt) and RQ (CO_2:O_2) increased with increasing O_3 exposure level. Seasonal patterns showed increased respiration rates during periods of rapid root growth in spring and early fall. Respiration quotient tended to decrease during known periods of active root growth in control seedlings, but a similar response was not observed in O_3-treated seedlings. Responses to O_3 were greatest in the soil-grown plants, which had a lower fertility level than media-grown plants. Although root d. wt was decreased, root:shoot ratios did not change in response to O_3. Soil-grown plants had higher root-shoot ratios than media-grown plants, reflecting the lower fertility of the soil.

Plant exposure to O_3 was found to affect both active and total populations of soil organisms. In both organic potting media and in soil, biomass of active soil fungi, and the ratio of active-fungal to active-bacterial biomass increased with increasing plant exposure to O_3. The effect of O_3 on total fungal and bacterial biomass was not linear: at low O_3 levels, total fungal and bacterial biomass increased; at the high O_3 level, total fungal and bacterial biomass decreased compared with those of controls.

Our results show that O_3 exposure to shoots significantly disrupts CO_2 production and O_2 consumption of soil and roots of ponderosa pine seedlings. Below-ground respiratory differences were thought to be a result of changes in respiratory substrates, carbon refixation within the plant and soil microbial activity. Ozone also changes below-ground RQ, suggesting that O_3 substantially disrupts root metabolism and interactions with rhizosphere organisms. Ozone exposure of ponderosa pine grown in different soil types can disrupt below-ground respiration and influence populations of soil organisms without alteration of biomass partitioning between above- and below-ground plant components. Collectively, the effect of O_3 on the below-ground system is of concern since it is likely that these changes are accompanied by a change in the ability of root systems to acquire nutrient and water resources and possibly to synthesize amino acids and proteins necessary for normal plant function.

Key words: O_3, ponderosa pine, root respiration, respiration quotient, microbial respiration.

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INTRODUCTION

Ozone (O_3) is known to affect tree growth adversely (Hogsett *et al.*, 1985*a*). In the past, emphasis has been placed on understanding relationships between photosynthesis (Clark *et al.*, 1995) and growth (Hogsett *et al.*, 1985*a*) in response to O_3, whereas little attention has been paid to respiratory processes. Respiratory losses can account for a large fraction of the carbon fixed by plants and can be a more important determination of yield (Pearcy *et al.*, 1987). Up to 50% of photosynthetically fixed C can be translocated below ground (Hendrick & Pregitzer, 1993) where as much as 25% can be lost through respiration (Andersen & Rygiewicz, 1995). Ozone differentially alters C partitioning as a function of both O_3 concentration and plant developmental stage (Cooley & Manning, 1987), and has been shown to decrease C allocation below ground and root growth (Tingey, 1974; Blum, Mrozek & Johnson, 1983; McCool & Menge, 1983; Adams *et al.*, 1990; Gorissen, Schelling & van Veen, 1991*a*; Karnosky *et al.*, 1996; Coleman *et al.*, 1995). The degree to which the effect of ozone on shoot physiology impairs root physiology and soil processes has not been fully determined.

Root respiration decreases or increases in response to above-ground O_3 exposure, depending on the experimental conditions (Hofstra *et al.*, 1981; Blum *et al.*, 1983; Ito *et al.*, 1985; Edwards, 1991; Nouchi *et al.*, 1991, 1995; Andersen & Scagel, 1997). Although root growth and activity are linked to shoot growth and photosynthesis, their seasonality is distinctly different from those of the shoot. Hanson *et al.* (1993) found that seasonal trends in soil CO_2 effluxes correlated with the magnitude of above-ground C fixation. Root activity and allocation changes throughout the season could result in differential root respiratory responses to O_3 at different times of the year.

Soil type has also been shown to influence plant response to O_3 (Reich *et al.*, 1986; Stroo *et al.*, 1988) and might be a major factor contributing to differential sensitivity to O_3. Soil nutrient limitations can intensify the physiological effects of O_3 while unlimited nutrient availability might reduce plant response to O_3 (Pell *et al.*, 1990; Gorissen, Joosten & Jansen, 1991*b*; Greitner, Pell & Winner, 1994; Pääkkönen & Holopainen, 1995). Andersen & Scagel (1997) found that below-ground respiratory fluxes of O_3-exposed ponderosa pine were increased under low-nutrient conditions.

The extent of O_3 impact on O_2 uptake and CO_2 release from roots and rhizosphere soil has not been investigated. The relationship between respiratory O_2 uptake and CO_2 release can be used to follow physiological shifts resulting from changes in environmental conditions and root metabolism and might be useful for following changes in soil microbial activity. CO_2 evolution from soil originates from soil microbial activity and respiration by roots. Ozone exposure of plants has been shown to decrease mycorrhizal fungal colonization of roots (McCool & Menge, 1983, 1984; Andersen & Rygiewicz, 1991) but little is known about the indirect effects of O_3 on other soil micro-organisms. Reduced C fixation in O_3-exposed plants, and resultant changes in below-ground C allocation and root turnover could lead to changes in soil microbial populations, which could ultimately influence total below-ground respiratory activity.

The purpose of the present study was to test the hypothesis that O_3 alters the seasonal patterns of fluxes of CO_2 and O_2 from roots and soil of ponderosa pine seedlings. Two soil types were employed; an organic potting medium amended with fertilizer, and a loamy-sand ponderosa pine soil without fertilizer amendment. The results are discussed in terms of seasonal-O_3 and soil-type responses as well as the influence of microbial activity.

MATERIALS AND METHODS

Seedling culture

Ponderosa pine (*Pinus ponderosa* Laws.) seedlings, grown from seeds originating from Butte County, CA, Zone 524 (U.S. Forest Service) at an elevation of 1066–1219 m, were obtained from a California Department of Forestry nursery (Magalia, CA) as cold-stored, bare-root 2+0 stock. Seedlings were transplanted into PVC pipe pots (15-cm diameter × 38-cm depth) containing either a 1:1 (v/v) mixture of Sunshine Mix no. 2 (SunGro Horticulture Inc., Bellevue, WA) and perlite (Supreme Perlite Co., Portland, OR) amended with 26 g of slow-release fertilizer (SIERRA 17–6–10 N, P, K Plus Minors, Grace-Sierra Horticultural Products Co., Milipitas, CA), or a pumiceous loamy-sand soil collected from a ponderosa pine site 10 km south-east of Bend, Oregon (Mapping Unit 6A, elevation 1219 m), and not amended with fertilizer.

Plants in loamy-sand soil (S) (average bulk density 1·33 g cm^{-3}) and plants in the organic potting media (M) (average bulk density of 0·94 g cm^{-3}) were grown for 1 month in an open-air nursery before the first year's O_3 exposure. Plants were kept in exposure chambers for 5 months (May–October 1993) before being moved into an open-air nursery for winter then returned to exposure chambers the following year (June 1994) until one of three harvest dates.

Harvests were taken in August 1994 (after full needle expansion during the second exposure season; harvest 1), October (at the end of second exposure season; harvest 2), and November (1 month after completion of the second exposure season; harvest 3). Seedlings were watered with tap-water as needed and pesticides were applied to control aphids

Table 1. *O_3 exposure values (SUM00 and SUM06) and duration of fumigation periods for each year of the ponderosa pine study*

	SUM00 and SUM06 ozone exposure (μmol mol^{-1} h^{-1})*					
	1993		1994†			
O_3 treatment	SUM00	SUM06	SUM00	SUM06	SUM00	SUM06
CF (control	23·7	0·0	30·9	0·0	36·1	0·0
Ep-23	112·1	37·6	173·7	56·9	213·1	69·8
Ep-31	153·1	87·0	237·3	133·2	293·5	163·8
Duration (d)	147		79		131	

* Mean values over two O_3 chamber replicates per O_3-treatment.
† 1994 values are cumulative over both exposure years.

(encapsulated Diazinon®) and spider mites (Avermectin® B_1 solution).

Ozone exposures

Seedlings were exposed for a total of 147 d in 1993 (31 May–13 October) and for 79 d (harvest 1; 7 June–6 August) or 131 d (harvests 2 and 3; 7 June–13 October) during 1994 in six modified open-top fumigation chambers in Corvallis, OR. Fumigations were monitored and controlled by an automated gaseous pollutant-exposure system (Hogsett, Tingey & Holman, 1985*b*). Charcoal-filtered air was supplied to each open-top chamber, and O_3 (PC1 Ozone Corporation ozone generator) was injected to the inlet air stream after filtration to raise concentrations to the desired level within each chamber. Ozone treatment profiles were developed to reflect ambient regional air quality from the Midwest United States (Lefohn, Hogsett & Tingey, 1986; 1987) and consisted of episodic patterns of varying daily peak concentrations on 28-d cycles as described by Clark *et al.* (1995). Three treatments were each replicated twice: CF (control, charcoal-filtered air without O_3 addition), Ep-23 (23 μmol mol^{-1} per cycle) and Ep-31 (31 μmol mol^{-1} per cycle, mean hourly concentration summed each hour over the entire exposure period). Total O_3 exposure values (SUM00) were calculated for each chamber by summing the hourly mean concentrations for 147 d in 1993 and either 79 or 131 d in 1994 (Table 1). For comparison, SUM06 levels are reported for each chamber by summing all hourly mean concentrations $\geqslant$ 0·06 ppm for 147 d in 1993 and either 79 or 131 d in 1994 (Lee, Hogsett & Tingey, 1988) (Table 1).

Morphological measurements

Total plant height and root collar diameter were measured every 4 wk from May to November 1993, and from March to November 1994. Total above-ground plant size was estimated by D^2H = ((root collar diameter)^2 × height). Root biomass was estimated based on regressions with D^2H from a subset of trees from each treatment, and was used to adjust below-ground gas exchange measures of sample trees for sample dates when plants were not destructively harvested. Trees were harvested in August, October and November 1994. At harvest, plants were divided into needle, stem, taproot and lateral root components and oven-dried weights were obtained.

Substrate microbial analyses

Representative substrate samples were taken from the loamy-sand soil and organic potting media at the beginning of the experiment (April 1993) and assayed for active and total fungal (AFB and TFB; biomass in μg g^{-1} soil d. wt) and bacterial (ABB and TBB; biomass in μg g^{-1} soil d. wt. tare) biomass. During the second harvest (October 1994) substrate samples were taken from a subsample of representative pots and assayed for TFB, AFB, TBB and ABB. Populations were estimated by dilution and counting using a light microscope; active hyphae and bacteria were identified using fluorescein diacetate (FDA) staining followed by direct microscopy (Lodge & Ingham, 1991).

Physiological instrumentation

Specially designed PVC pot covers (similar to that described in Edwards, 1991) were used to measure CO_2 and O_2 fluxes from the soil surface using a Micro-Oxymax 4·2 System with CO_2 and O_2 sensors (Columbus Instruments, Columbus, OH). The Micro-Oxymax System is a closed system designed to detect very low levels of O_2 consumption and CO_2 production and to calculate RQ of sequential samples. Air in the enclosed headspace above the soil surface is pumped through the gas sensors where the gas fractions are measured and returned to the pot headspace. Changes in the levels are used to compute O_2 consumption and CO_2 production (normalized to

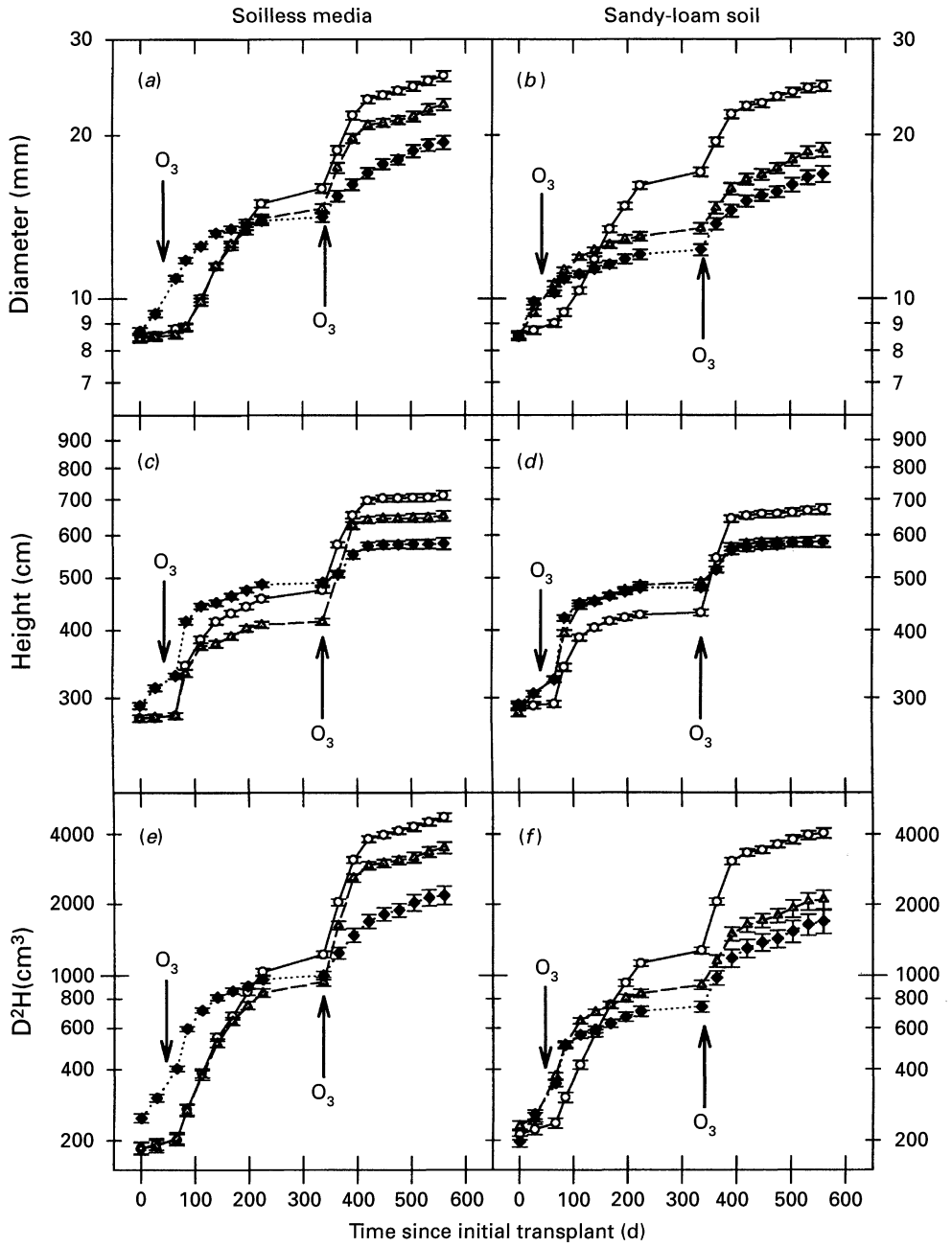

Figure 1. Root collar diameter (*a*, *b*), total plant height (*c*, *d*) and above-ground plant size (D^2H) (*e*, *f*) of ponderosa pine trees over two growing seasons' exposure to O_3. Geometric LS means and SE are plotted on a $\log_{10}$ *y*-axis (chamber $n = 2$/ozone level for each date; total plant number varies by date, see 'Materials and Methods'). (*a*), (*c*), (*e*) Influence of O_3 exposure on pine growing in organic potting media. (*b*), (*d*), (*f*) Influence of O_3 exposure on pine growing in sanding-loam soil. ○, charcoal-filtered control; △, Ep-23 O_3 exposure; ◆, Ep-31 O_3 exposure. Arrows indicate timing of O_3 exposure initiation in early June of 1993 and 1994.

O °C and 760 mm Hg) by the software run in the controller. A reference chamber is used to recalibrate the sensors before each measurement using standard gases. The oxygen sensor operates as an oxygen battery (fuel cell) measuring O_2 percentage directly, and the CO_2 sensor is a single-beam non-dispersive infra-red device. The system is fully automated utilizing microcomputer and an expansion interface with 20 channels for sequential measurements of up to 20 plants at one time.

To measure below-ground gas fluxes, PVC enclosures were attached to pots containing trees by encircling the stem of each tree with enough closed-cell foam to create a tight seal between the stem and the hole in the center of the top of the PVC enclosure. Closed-cell foam seams on the top of each PVC enclosure were pressed together and held in place with spring-loaded clamps attached to the base of the PVC enclosure encircling the top of the pot. Two hose connectors on the top of the PVC

Table 2. *Root collar diameter, total height and total above-ground plant size (D^2H) of O_3-exposed ponderosa pine growing in two different substrates March 1993–November 1994*

Factor	Repeated measures variables ($P > F$ values)*		
	Diameter	Height	D^2H
O_3	0·007	0·740	0·039
Substrate	0·026	0·661	0·013
Time	0·000	0·000	0·000
O_3 × time	0·000	0·017	0·000
Substrate × time	0·000	0·000	0·000
O_3 × substrate × time	0·024	0·939	0·573

* Analysis of harvest 3 plants only (chamber n = 2/ozone level; 48 plants total).

MANOVA results of morphological variables treated as repeated measures (probability > F values).

enclosure were used for plumbing the enclosure to the Micro-Oxymax system. Sampling volume of each pot was measured automatically by the System.

Below-ground respiration measurements

Measurements of CO_2 and O_2 flux rates and RQ (respiratory quotient CO_2:O_2; Minchin & Witty, 1990) from the soil surface of pots were obtained at monthly intervals from May to June 1994 during the second O_3 exposure season. Soil temperature was measured by a hand-held temperature probe, and soil moisture content was measured using time domain reflectometry (Brisco *et al.*, 1992).

At each harvest date, CO_2 and O_2 flux rates and RQ were measured on a subsample of plants, which were then detopped, and pots containing roots and soil were placed in a 5 °C cooler. After 7 d at 5 °C, CO_2 and O_2 flux rates and RQ were measured again on the pots to obtain an estimate of below-ground maintenance respiration (Marshall & Perry, 1987).

Statistical analyses

The study used a split-plot design with whole-plot treatments of three O_3 levels (control, Ep-23 and Ep-31) randomized within two chambers per O_3 level.

Table 3. *Ponderosa pine biomass responses to O_3 exposure and growing substrate, and summary of ANOVA results (LS means and probability > F values), by harvest*

Factor* (Harvest date)	Substrate type	O_3 exposure level	Harvest dry weight (g) and ratio probabilities ($P > F$)					
			Needle	Stem	Above-ground	Total root	Total plant	Root:shoot
Means (August) (g)	Organic potting medium	Control	62·7	74·8	138·3	56·5	195·0	0·41
		Ep-23	52·1	50·5	104·0	38·6	142·9	0·37
		Ep-31	55·8	51·6	107·7	38·7	146·8	0·36
	Sandy-loam soil	Control	19·6	17·6	37·6	21·6	60·6	0·61
		Ep-23	18·6	18·5	37·3	20·9	58·7	0·57
		Ep-31	16·3	15·8	32·1	16·8	49·4	0·54
$P > F$	O_3		0·374	0·099	0·109	0·185	0·084	0·672
	SUM00-linear†		0·190	0·045	0·050	0·088	0·038	0·411
	Substrate		0·000	0·000	0·000	0·000	0·000	0·000
	O_3 × substrate		0·567	0·027	0·161	0·127	0·122	0·978
Means (October) (g)	Organic potting medium	Control	54·4	83·5	129·4	75·9	205·8	0·59
		Ep-23	42·3	87·7	131·3	70·2	202·4	0·54
		Ep-31	35·5	98·1	134·2	66·9	201·8	0·50
	Sandy-loam soil	Control	20·9	27·6	43·8	34·1	78·3	0·79
		Ep-23	11·0	26·8	40·5	28·9	70·7	0·74
		Ep-31	7·5	26·1	30·4	28·1	58·9	0·94
$P > F$	O_3		0·029	0·853	0·486	0·322	0·449	0·577
	SUM00-linear		0·018	0·656	0·331	0·164	0·263	0·909
	Substrate		0·000	0·000	0·000	0·000	0·000	0·000
	O_3 × substrate		0·026	0·564	0·269	0·892	0·447	0·133
Means (November) (g)	Organic potting medium	Control	44·2	103·5	158·9	83·8	243·5	0·51
		Ep-23	42·8	96·0	139·8	77·9	218·2	0·63
		Ep-31	30·9	80·9	112·7	64·8	178·4	0·62
	Sandy-loam soil	Control	15·9	39·1	60·2	41·9	102·4	0·77
		Ep-23	9·5	38·3	51·4	41·3	92·8	0·81
		Ep-31	3·5	34·9	43·2	31·5	74·9	0·95
$P > F$	O_3		0·034	0·270	0·097	0·023	0·079	0·222
	SUM00-linear		0·015	0·167	0·049	0·012	0·044	0·165
	Substrate		0·000	0·000	0·000	0·000	0·000	0·000
	O_3 × substrate		0·482	0·768	0·975	0·853	0·997	0·479

* Analyses for each harvest date separately (chamber n = 2/per ozone level; 48 plants per harvest).

† SUM00 refer to contrasts based on exposure indices given in Table 1.

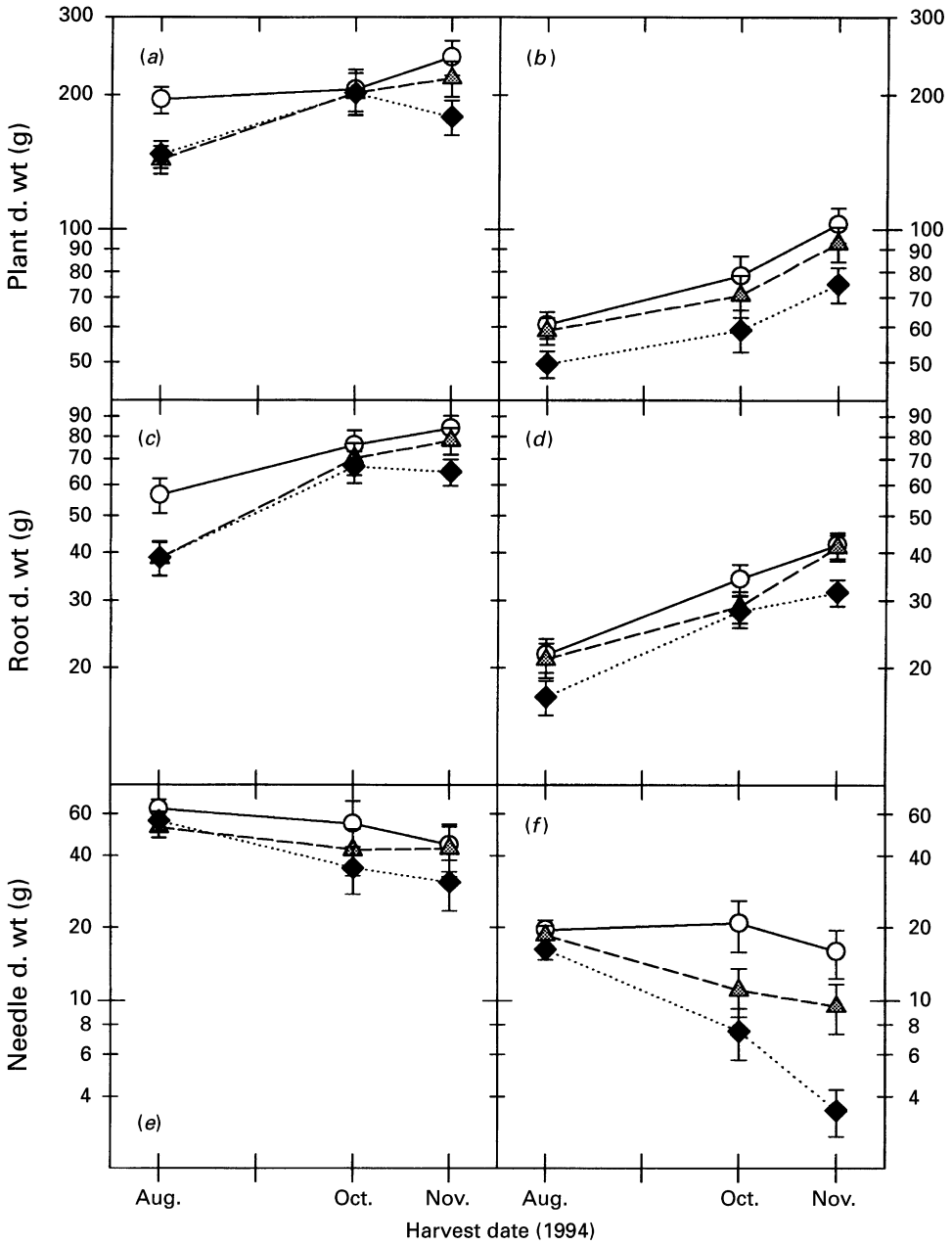

Figure 2. Total plant dry weight (*a*, *b*), root d. wt (*c*, *d*) and needle d. wt (*e*, *f*) of ponderosa pine trees after two growing seasons' exposure to O_3. Geometric LS means and SE are plotted on a $\log_{10}$ *y*-axis (chamber n = 2/ozone level; 48 plants in total for each date). (*a*), (*c*), (*e*) Influence of O_3 exposure on d. wt of pine growing in organic potting media. (*b*), *d*), (*f*) Influence of O_3 exposure on d. wt of pine growing in sandy-loam soil. ○, charcoal-filtered control; ◬, Ep-23 O_3 exposure; ◆, Ep-31 O_3 exposure.

Sub-plot treatments included the two substrate types, loamy-sand soil (S) and organic potting media (M), with 12 plants per substrate treatment per chamber, for a total of 144 plants. The unit of replication was the chamber, and all data analyses were performed using SAS (1990). Data were log-transformed (i.e. $y_i' = \log_e (y+1)$) to reduce the heterogeneity of variances detected between treatment groups by Bartlett's Test (Bartlett, 1937).

Analysis of variance (ANOVA) was performed for all respiration, morphological and microbial data separately for each measurement date. Orthogonal polynomial contrasts based upon the SUM00 O_3 were used to test for trends in the effects of O_3 on plant response. Least squares means were calculated for the O_2 main effects, substrate (S vs. M) main effects, and separate O_3 substrate interactions.

Repeated measurements of O_2 and CO_2 fluxes and RQ taken at pre-harvest and post-harvest dates were analysed using multivariate ANOVA (MANOVA) to test for O_3 and fertility effects and their interaction. Post-MANOVA tests included soil-type main effects and orthogonal polynomial contrasts for O_3 main effects to detect linear and quadratic trends in plant responses.

Repeated measures analysis was done on res-

piration and morphological data over the course of the experiment. Analyses included univariate ANOVA for between-plant effects (O_3 and substrate), univariate ANOVA for within-plant effects (measurements at different dates) and interactions between date × O_3 × substrate, and multivariate ANOVA (MANOVA) considering profile patterns in the response variable over time. Ozone treatment sum-of-squares were partitioned into linear and quadratic contrasts based upon the SUM00 O_3 indices. Multivariate tests were based upon the residual sums-of-squares-and-cross-products (SSCP) matrix rather than a chamber (O_3) SSCP matrix owing to insufficient degrees of freedom.

RESULTS

Morphological responses

Over the course of the experiment, O_3 reduced plant height, diameter and above-ground size (D^2H) as much as 11 % compared with controls (Fig. 1). The extent of these reductions was found to be dependant upon substrate and time (Table 2). Repeated measures analysis showed significant O_3 × time and substrate × time interactions for all three variables (Table 2). Although O_3-induced reductions in plant height, diameter and D^2H tended to be greatest in plants growing in organic potting media (Fig. 1), only root collar diameter showed a significant three-way interaction between O_3 × substrate × time (Table 2).

Harvest data from the three dates in 1994 (August, during the second exposure season, October, at the end of the second exposure season and November, after the second exposure season) indicated that substrate significantly affected d. wt of all plant parts (needles, stems and roots), while O_3 effects were both date- and tissue-type specific (Table 3). Soil-grown plants had significantly less d. wt in all plant parts when compared with media-grown plants (Fig. 2). Ozone had no effect on needle d. wt of plants harvested in August; however, in October, it significantly decreased needle d. wt of soil-grown plants more than in media-grown plants (Fig. 2*e*, *f*) and in November it significantly decreased needle d. wt of plants in both substrates. SUM00-linear contrasts were significant at these dates (Table 3).

Ozone significantly decreased root d. wt in November (Fig. 2*c*, *d*) but root:shoot ratios were not significantly influenced by O_3 at any of the three harvest dates (Table 3). As with other dry weights significantly affected by O_3, only SUM00-linear and contrasts were significant.

Microbial responses

Total fungal biomass (TFB) and total bacterial biomass (TBB) were significantly influenced by O_3 exposure (Table 4). Contrasts based upon SUM00-quadratic were significant, means for the Ep-23 treatment being higher than those for either the control or Ep-31. AFB tended to increase with increasing O_3 treatment, but differences were not significant, owing to high chamber variation. Substrate had a significant effect on AFB, TFB, active bacterial biomass (ABB) and TBB. In general, the sandy-loam soil had significantly higher levels of AFB while the organic potting media had significantly higher levels of ABB, TBB and TFB. There were no significant interactions between O_3 and substrate on microbial populations.

Respiration responses

For all sampling dates, CO_2 production (μmol h^{-1} g^{-1} total root d. wt) and O_2 consumption

Table 4. *Biomass responses of soil micro-organisms from two different growing substrates containing ponderosa pine exposed to different levels of O_3, and summary of ANOVA results (LS means and probability > ⩾ values)*

Factor*	Substrate type	O_3 exposure level	Fungal biomass (μg per g soil) Active	Fungal biomass (μg per g soil) Total	Bacterial biomass (μg per g soil) Active	Bacterial biomass (μg per g soil) Total
Means	Organic potting medium	Control	5·69	774	27·9	59·3
		Ep-23	15·30	2110	30·8	85·0
		Ep-31	17·90	904	24·1	38·6
	Sandy-loam soil	Control	26·40	404	21·0	32·9
		Ep-23	33·50	1062	18·6	45·9
		Ep-31	48·00	442	20·6	29·1
P > *F*	O_3		0·489	0·067	0·855	0·031
	SUM00-linear†		0·269	0·295	0·675	0·309
	SUM00-quadratic†		0·999	0·035	0·755	0·015
	Substrate		0·000	0·000	0·001	0·000
	O_3 × substrate		0·604	0·948	0·300	0·205

* ANOVA of Harvest 2 plants only (chamber n = 2/ozone level; 48 plants in total).
† SUM00 refer to contrasts based on exposure indices given in Table 1.

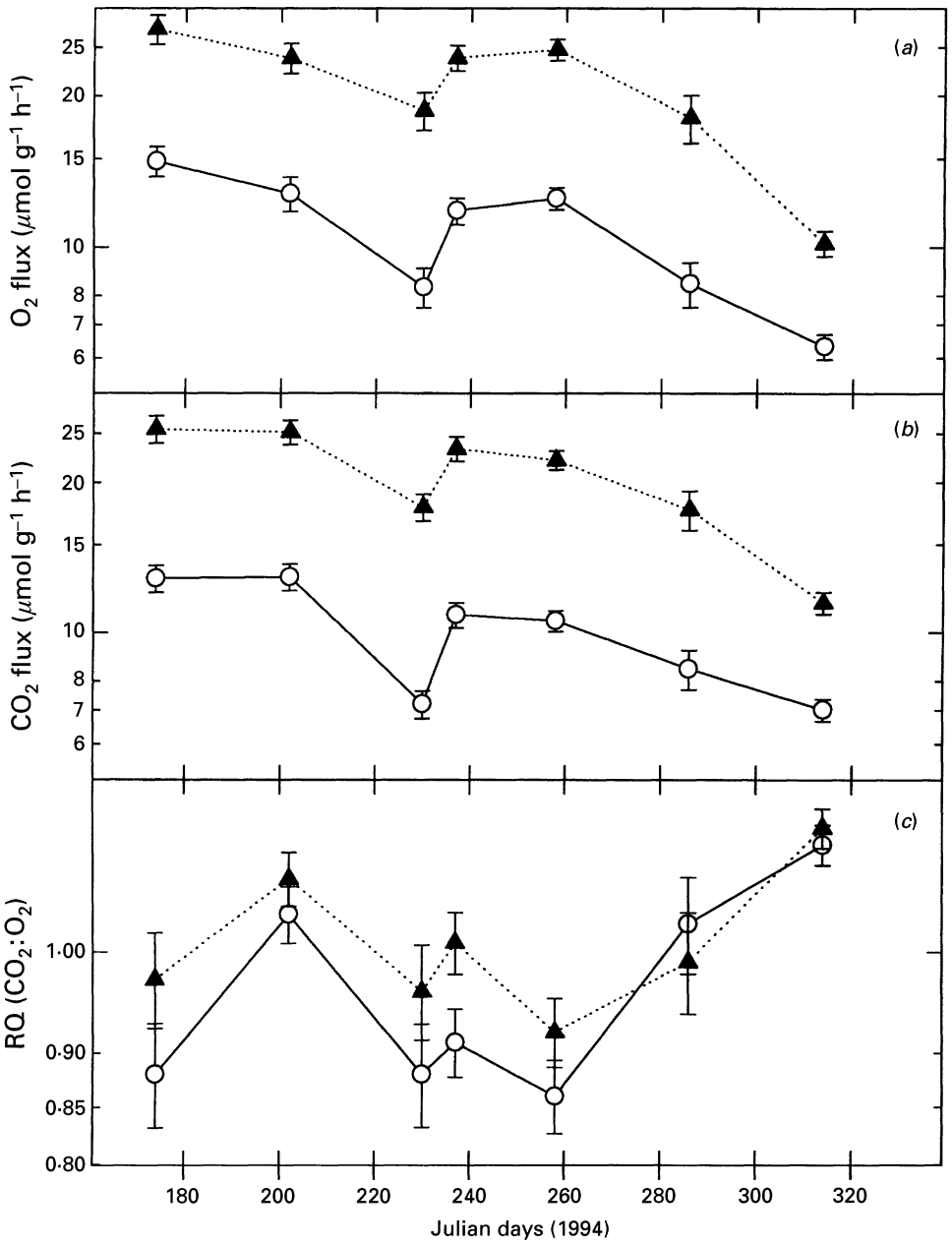

Figure 3. Influence of substrate type (○, organic potting media; ▲, sandy-loam soil) on below-ground respiratory activity of ponderosa pine trees during the 1994 growing season (May–November). Geometric LS means and SE (over all O_3 exposure levels) are plotted on a $\log_{10}$ y-axis (chamber $n = 2$/ozone level for each date; total plant number varies by date, see materials and methods). (*a*), O_2 flux. (*b*) CO_2 flux. (*c*) RQ.

(μmol h^{-1} g^{-1} total root d. wt) were lower in organic potting media than in sandy-loam soil (Fig. 3*a*, *b*). *P*-values from ANOVA of log-transformed respiration data expressed on a root d. wt basis are given in Table 5. Ozone tended to have a quadratic influence on respiratory activity in the fall of 1993, with Ep-23 having higher CO_2 production and O_2 consumption than control and Ep-31-treated plants (Table 5). The remainder of the measurements showed a linear response to O_3 treatment, with Ep-31-treated plants having higher CO_2 production and O_2 consumption than control and Ep-23-treated plants (Fig. 4*a*, *b*). Significant $O_3 \times$ substrate interactions occurred July–September 1994 for both O_2 consumption and CO_2 production (Table 5). Generally, both O_2 consumption and CO_2 production increased in response to O_3 exposure of sandy-loam grown plants, whereas organic potting media plants were less affected (Fig. 5*a*, *b*, *d*, *e*).

RQ values were significantly lower in organic potting medium than in sandy-loam soil for six out of eight sampling dates (Figure 3*c*). *P*-values from ANOVA of log-transformed RQ values are given in Table 5. RQ values for five out of eight sampling dates tended to be higher in O_3 treatments; however, this trend was only significant in June, July and August (Fig. 4*c*; Table 5). The response of RQ values to O_3 was not significantly different between

Table 5. *Below-ground respiratory responses (CO_2 flux, O_2 flux and RQ) September 1993–November 1994 for O_3-exposed ponderosa pine growing in two different substrates, and summary of* ANOVA *results (LS means and probability > F values)*

		Organic potting media			Sandy-loam soil			ANOVA results§			
Variable	Date*	CF†	Ep-23	Ep-31	CF	Ep-23	Ep-31	O_3‡	SUM00 linear/quadratic	S	$O_3 \times S$
O_2 flux (μmol g^{-1})	SEP93	66·6	67·5	47·8	50·7	74·6	33·1	0·015	0·052/0·009	0·040	0·067
	JUN94	16·2	13·3	15·1	29·0	27·0	25·7	0·484	0·336/0·506	0·000	0·584
	JUL94	11·9	13·5	12·9	24·2	18·5	30·0	0·097	0·210/0·060	0·000	0·007
	AUG94	10·0	8·0	7·2	16·4	14·5	27·7	0·298	0·742/0·154	0·000	0·000
	AUG94	11·4	11·1	13·1	19·8	18·9	36·1	0·069	0·078/0·068	0·000	0·001
	SEP94	12·7	12·2	12·7	18·2	23·2	35·8	0·078	0·045/0·215	0·000	0·002
	OCT94	7·8	8·1	9·5	13·1	17·1	26·3	0·111	0·062/0·307	0·000	0·116
	NOV94	5·5	6·8	6·8	9·5	10·7	10·4	0·623	0·395/0·741	0·000	0·857
CO_2 flux (μmol g^{-1})	SEP93	58·2	60·0	43·0	55·0	67·5	34·6	0·023	0·046/0·018	0·500	0·196
	JUN94	11·8	12·4	14·4	24·0	24·8	27·9	0·111	0·072/0·210	0·000	0·963
	JUL94	11·5	13·7	13·6	23·3	20·8	32·8	0·042	0·033/0·068	0·000	0·007
	AUG94	7·1	7·5	7·0	13·7	13·4	30·7	0·076	0·068/0·094	0·000	0·000
	AUG94	10·6	10·5	11·5	19·2	19·2	34·4	0·048	0·048/0·056	0·000	0·000
	SEP94	10·7	10·7	10·3	17·8	20·6	29·6	0·113	0·067/0·264	0·000	0·002
	OCT94	7·8	8·0	9·7	13·8	16·0	24·7	0·061	0·040/0·131	0·000	0·196
	NOV94	6·2	7·3	7·7	10·5	11·8	12·2	0·574	0·332/0·918	0·000	0·959
RQ	SEP93	0·90	0·93	0·93	1·10	0·95	1·05	0·639	0·808/0·395	0·003	0·172
	JUN94	0·75	0·95	0·98	0·84	0·93	1·11	0·047	0·025/0·682	0·033	0·194
	JUL94	1·00	1·03	1·08	0·99	1·14	1·12	0·017	0·007/0·269	0·050	0·081
	AUG94	0·78	0·95	0·98	0·85	0·93	1·09	0·051	0·023/0·655	0·028	0·182
	AUG94	0·90	0·97	0·88	0·98	10·4	1·03	0·564	0·636/0·367	0·000	0·421
	SEP94	0·86	0·90	0·85	0·99	0·92	0·88	0·319	0·197/0·463	0·095	0·412
	OCT94	1·04	1·02	1·03	1·07	0·94	0·97	0·496	0·342/0·522	0·357	0·524
	NOV94	1·13	1·10	1·14	1·12	1·12	1·19	0·783	0·717/0·585	0·437	0·576

* SEP93, 30 September 1993; JUN94, 23 June; JUL94, 21 July; AUG94, 8 August or 21 August; SEP94, 15 September; OCT94, 13 October; NOV94, 10 November 1994.

† CF, Charcoal-filtered controls; Ep-23, Episodic-23 O_3; Ep-31, Episodic-31 O_3.

‡ ANOVA probabilities ($P > F$) for main effects O_3 and substrate (S), interactions ($O_3 \times S$) and SUM00 contrasts based on indices in Table 1.

§ Analysis of Harvest 3 plants only (chamber $n = 2$/ozone level; 48 plants in total).

substrates for any of the measurement times (Table 5; Fig. 5*c*,*f*).

Below-ground respiration responses expressed on the basis of soil surface area (μmol m^{-2} h^{-1}) and root d. wt (μmol g^{-1}) are given in Table 6. Ozone, substrate and seasonal changes in respiration expressed on a soil surface area basis were similar to those found when CO_2 production and O_2 consumption were expressed on a root d. wt basis.

Seasonal trends in respiration responses

The effects of O_3 and substrate type on seasonal changes in respiration varied with time of year and parameter measured (CO_2 flux, O_2 flux or RQ values) (Fig. 5). Over the course of the experiment, O_3- and substrate-induced changes in CO_2 and O_2 fluxes varied significantly by date (date × O_3 × substrate interaction), whereas only O_3-induced changes in RQ values varied by date (O_3 × date interaction). *P*-values from repeated measures analysis performed on respiratory-response data from harvest 3 plants during the 1994 growing season are shown in Table 7.

In general, during the 1994 growing season, plants growing in sandy-loam soil had higher CO_2 and O_2 fluxes and exhibited a greater sensitivity to O_3 exposure and a greater seasonal periodicity (Fig. 5). MANOVA indicated significant date × O_3 × substrate interactions for CO_2 flux and O_2 flux, whereas RQ values were only significantly influenced by the interaction between date and O_3 exposure. *F*-values based on MANOVA using the residual SSCP matrix and Pillai's Trace are reported in Table 8.

Comparison of pre- and post-harvest respiration responses

Detopping (and cold storage for 7 d) had less of an effect on respiratory activity of O_3-exposed plants than on that of CF controls, and less of an influence on sandy-loam than on organic potting-media-grown plants. When treated as repeated-measures data,

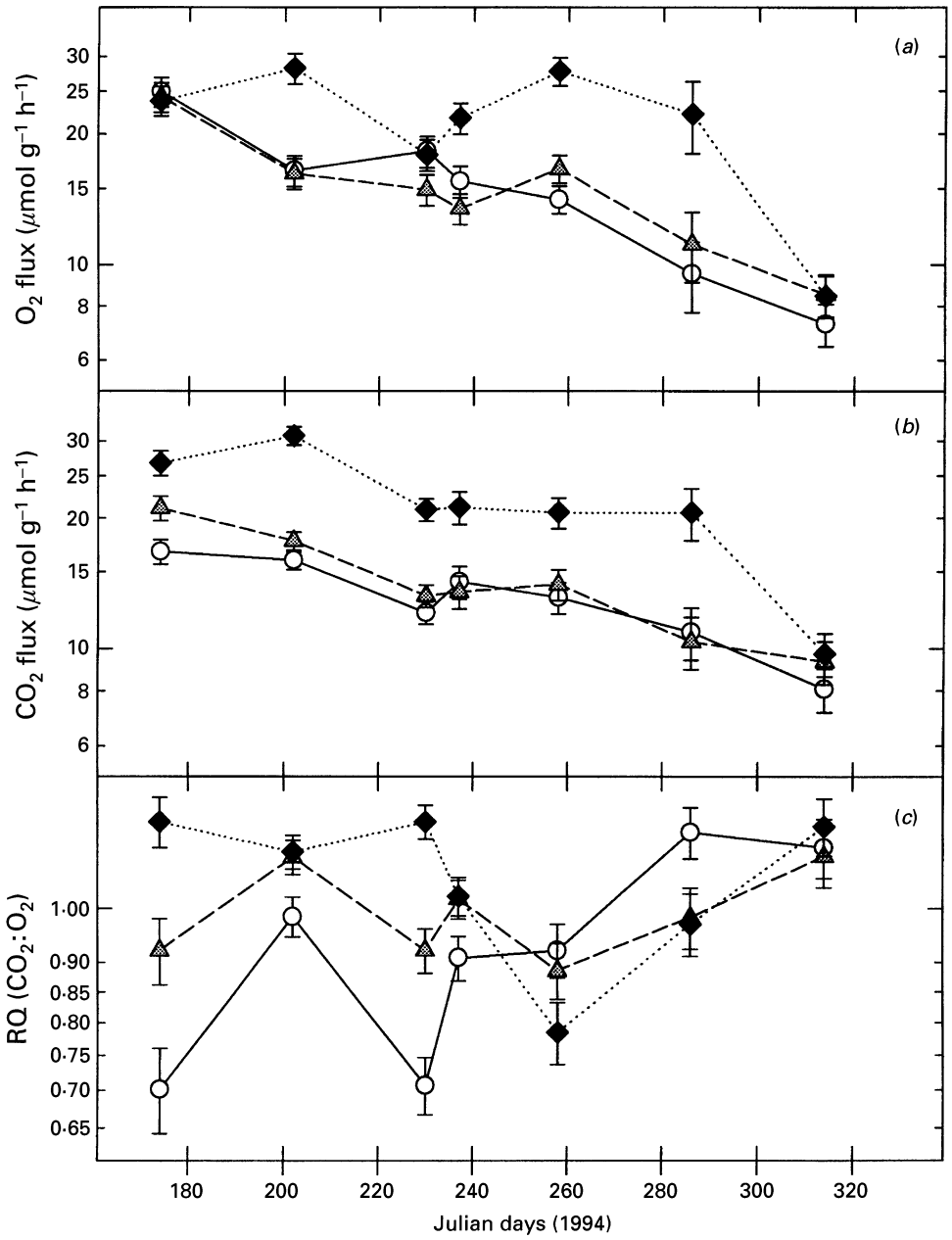

Figure 4. Influence of O_3 exposure (○, charcoal-filtered control; △, Ep-23 O_3 exposure; ◆, Ep-31 O_3 exposure) on below-ground respiratory activity of ponderosa pine trees during the 1994 growing season (May–November). Geometric LS means and SE (over both substrate types) are plotted on a $\log_{10}$ y-axis (chamber $n = 2$/ozone level for each date; total plant number varies by date, see 'Materials and Methods'). (*a*) O_2 flux. (*b*) CO_2 flux. (*c*) RQ.

MANOVA detected significant time × O_3 × substrate interactions for CO_2 production and O_2 consumption, with O_3 increasing the response in organic potting-media plants and decreasing the response in sandy-loam plants (Table 9). A significant time × O_3 interaction was detected for RQ, with RQ values increasing between pre- and post-harvest more in CF controls than O_3-treated plants (Table 9).

We also found seasonal changes in respiration responses to detopping. In August, during the second O_3 exposure season, post-harvest CO_2 production, O_2 consumption and RQ were higher than pre-harvest (Fig. 6, sandy-loam soil). In October 1994, at the end of the second O_3 exposure season, post-harvest CO_2 production and O_2 consumption were similar to before harvest, whereas RQ decreased (Fig. 6, sandy-loam soil). In November 1994, after the second O_3 exposure season, post-harvest CO_2 production, O_2 consumption and RQ were lower than pre-harvest (Fig. 6, sandy-loam soil). The magnitude of these responses varied with O_3 treatment and substrate. MANOVA results of pre- and post-harvest respiration responses are given in Table 9.

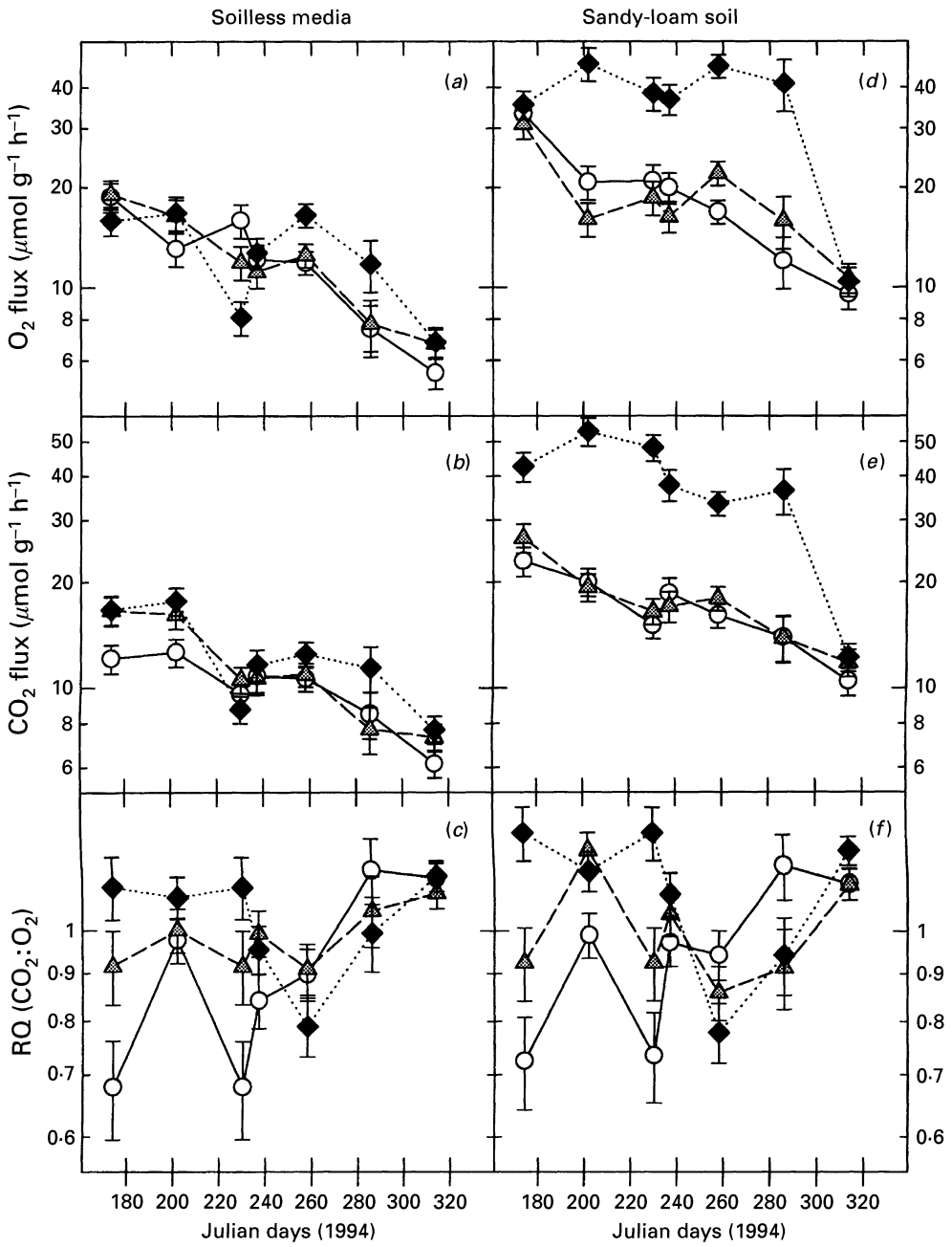

Figure 5. Influence of O_3 exposure (○, charcoal-filtered control; △, Ep-23 O_3 exposure; ◆, Ep-31 O_3 exposure) and substrate type (M, organic potting media; S, sandy-loam soil) on below-ground respiratory responses of ponderosa pine trees during the 1994 growing seasons. Geometric LS means and SE are plotted on a $\log_{10}$ y-axis (chamber $n = 2$/ozone level for each date; total plant number varies by date, see 'Materials and Methods'). (*a*)–(*c*) Influence of O_3 exposure on O_2 (*a*) CO_2 (*b*) fluxes and RQ (*c*) of pine in media (M). (*d*)–(*f*) Influence of O_3 exposure on O_2 (*d*) CO_2 (*e*) fluxes and RQ (*f*) of pine in soil (S).

DISCUSSION

In our study, substrate type had a significant effect on respiration, with the higher organic matter, higher fertility organic potting medium having much lower respiration rates than the sandy-loam soil (Fig. 3). Lower soil fertility was found to increase soil-root respiration rates of ponderosa pine in a previous study (Andersen & Scagel, 1997). The instrumentation and techniques used to measure respiratory responses in this study recorded CO_2 efflux rates (10–50 μmol g^{-1} h^{-1}; 6–20 μmol m^{-2} s^{-1}) similar to those observed by others measuring root and total below-ground CO_2 respiration. Ewel, Cropper & Gholz (1987) quantified root respiration in slash pine (*Pinus ellottii*) plantations as the difference between soil CO_2 evolution and the sum of the other major processes (decomposition of litter, fine roots, and fine particles of soil organic matter). The magnitude of our estimates (expressed on a soil surface area basis) approximates measurements they obtained from the forest floor of a young pine stand. Although

Table 6. *Below-ground respiratory responses in July and October 1994 for O_3-exposed ponderosa pine growing in two different substrates: summary of ANOVA results (probability > F values)**

		Respiratory response variable probabilities ($P > F$ values)					
		O_2		CO_2		RQ	
Expression	Factor	July	Oct.	July	Oct.	July	Oct.
μmol m^{-2} soil surface	O_3	0·026	0·787	0·061	0·595	0·017	0·496
	SUM00-linear†	0·012	0·754	0·028	0·390	0·008	0·342
	SUM00-quadratic	0·260	0·571	0·516	0·661	0·269	0·522
	Substrate	0·000	0·462	0·001	0·164	0·056	0·357
	O_3 × substrate	0·090	0·344	0·141	0·160	0·081	0·524
μmol g^{-1} root d. wt	O_3	0·097	0·111	0·042	0·061		
	SUM00-linear	0·210	0·032	0·033	0·040		
	SUM00-quadratic	0·060	0·307	0·067	0·131		
	Substrate	0·000	0·000	0·000	0·000		
	O_3 × substrate	0·007	0·115	0·007	0·195		

* Analysis based on Harvest 3 plants only (chamber $n = 2$/ozone level; 48 plants in total).
† SUM00 refers to contrasts based on exposure indices given in Table 1.

Table 7. *Below-ground respiratory responses (μmol g^{-1} root d. wt) of O_3-exposed ponderosa pine growing in two different substrates: summary of results from univariate ANOVA contrasts among the effects of date, O_3 and substrate (probability > F values)*

		Respiratory response variable probabilities ($P > F$ values)‡					
Response variable	Factor	June–July*	July–Aug.	Aug.–Aug.	Aug.–Sept.	Sept.–Oct.	Oct.–Nov.
O_2 (μmol g^{-1})	Mean	0·000	0·019	0·734	0·013	0·000	0·000
	O_3	0·007	0·004	0·210	0·018	0·633	0·023
	SUM00-linear†	0·007	0·002	0·145	0·008	0·509	0·022
	SUM00-quadratic	0·010	0·023	0·291	0·299	0·526	0·031
	Substrate	0·204	0·039	0·368	0·935	0·232	0·028
	O_3 × substrate	0·017	0·046	0·068	0·344	0·936	0·062
CO_2 (μmol g^{-1})	Mean	0·350	0·000	0·173	0·529	0·008	0·000
	O_3	0·002	0·111	0·400	0·562	0·191	0·046
	SUM00-linear	0·010	0·083	0·222	0·509	0·386	0·101
	SUM00-quadratic	0·001	0·163	0·713	0·425	0·111	0·031
	Substrate	0·077	0·000	0·155	0·217	0·340	0·056
	O_3 × substrate	0·003	0·005	0·018	0·497	0·915	0·077
RQ	Mean	0·000	0·019	0·734	0·013	0·000	0·000
	O_3	0·007	0·004	0·210	0·018	0·633	0·023
	SUM00-linear	0·007	0·002	0·145	0·008	0·509	0·023
	SUM00-quadratic	0·010	0·023	0·291	0·299	0·526	0·031
	Substrate	0·204	0·039	0·368	0·934	0·232	0·027
	O_3 × substrate	0·017	0·046	0·068	0·344	0·936	0·062

* Contrast dates: June–July = 23 June vs. 21 July; July–Aug. = 21 July vs. 8 August; Aug.–Aug. = 8 August vs. 21 August; Aug.–Sept. = 21 August vs. 15 September; Sept.–Oct. = 15 September vs. 13 October; Oct.–Nov. = 13 October vs. 10 November.
† SUM00 refers to contrasts based on exposure indices given in Table 1.
‡ Analysis of Harvest 1, 2 and 3 plants for June–July, July–Aug. dates (144 plants in total); Harvest 2 and 3 plants for Aug.–Aug., Aug.–Sept., Sept.–Oct. dates (96 plants in total); Harvest 3 plants for Oct.–Nov. (chamber $n = 2$/ozone level; 48 plants in total).

rates of below-ground and root CO_2 production have been well documented, few studies have reported O_2 consumption rates and RQ values so that the total respiratory response of the roots and soil could be examined.

Our results show seasonal changes in soil respiratory patterns. In our study, measurements were made throughout the season at approximately the same soil moisture contents and temperature. Although soil moisture content and temperature were monitored at the measurement time and used as covariates in initial ANOVAs of respiration data, these covariates were found to be insignificant in relation to our measurements (unpublished data). Seasonal patterns of CO_2 flux reported here are similar to those reported by Ewel *et al.* (1987), with CO_2 flux

Table 8. *Below-ground respiratory responses ($\mu mol\ g^{-1}$ root d. wt) of O_3-exposed ponderosa pine growing in two different substrates*

	Response variable probabilities ($P > F$ values)		
Factor*	O_2	CO_2	RQ
Dates	0·0001	0·0001	0·0001
Dates × O_3	0·0005	0·0022	0·0001
Dates × substrate	0·0001	0·0009	0·2366
Dates × O_3 × substrate	0·0012	0·0001	0·6473

* Analysis of Harvest 3 plants only (chamber n = 2/ozone level; 48 plants in total).

MANOVA results of respiration data treated as repeated measures (probability > F values).

increasing at times of greatest root growth in spring and early fall (Figs 3–5).

Below-ground respiratory activity was highest at the end of the first O_3-exposure season, and decreased during the 1994 exposure season (Fig. 4). Our measurements were taken from the exposed top portion of the soil in the pots. Root harvests revealed that the area in the top 5 cm of the substrate contained a high proportion of fine young roots and a large biomass component in large-diameter older structural roots. Small-diameter roots have high respiration rates per unit biomass (Barnard & Jorgensen, 1977) and root respiration decreases with root age (Palta & Nobel, 1989). It is possible that the influence of the large structural roots on our measurements might make our actual values of CO_2 and O_2 fluxes an underestimate of average root respiratory activity. Ledig, Drew & Clark (1976) reviewed 14 papers reporting root respiration rates of woody plants, primarily collected from excavated, detached root systems of seedlings, and reported rates of CO_2 flux ranging from 9 to 90 $\mu mol\ g^{-1}\ h^{-1}$. Rygiewicz & Andersen (1994) found *in situ* respiration rates of ponderosa pine seedlings ranging from 20 to 45 $\mu mol\ CO_2\ g^{-1}\ h^{-1}$. Cropper & Gholz (1991) estimated fine-root respiration rate at around 8·86 $\mu mol\ g^{-1}\ h^{-1}$ on a small, exposed portion of a

Table 9. *Pre- and post-harvest below-ground respiratory responses ($\mu mol\ g^{-1}$ root d. wt) of O_3-exposed ponderosa pine growing in two different substrates*

			O_2		CO_2		RQ	
Factor* (Date)	Substrate type	O_3 exposure level	Pre†	Post	Pre	Post	Pre	Post
Means (August)	Organic potting medium	Control	9·79	10·44	6·41	10·75	0·67	0·98
		Ep-23	8·57	12·94	7·91	15·09	0·93	0·86
		Ep-31	6·05	13·64	5·01	14·54	0·83	0·94
	Sandy-loam soil	Control	16·39	33·12	13·27	28·83	0·82	1·08
		Ep-23	13·09	21·41	11·51	24·15	0·88	0·90
		Ep-31	17·55	19·08	16·86	20·55	0·97	0·95
$P < F$	Time × O_3		0·647		0·863		0·269	
	SUM00-linear†		0·404		0·997		0·053	
	Time × substrate		0·000		0·000		0·003	
	Time × O_3 × substrate		0·005		0·003		0·013	
Means (October)	Organic potting medium	Control	8·23	8·04	7·26	7·36	0·92	0·96
		Ep-23	8·48	10·80	8·31	6·13	0·98	0·60
		Ep-31	7·62	11·48	8·16	8·27	1·07	0·78
	Sandy-loam soil	Control	14·18	13·85	13·72	12·81	0·97	1·01
		Ep-23	18·39	27·83	18·55	14·25	0·96	0·63
		Ep-31	16·78	21·59	16·61	14·08	0·98	0·77
$P < F$	Time × O_3		0·636		0·372		0·052	
	SUM00-linear		0·597		0·376		0·027	
	Time × substrate		0·000		0·000		0·560	
	Time × O_3 × substrate		0·253		0·347		0·591	
Means (November)	Organic potting medium	Control	5·49	4·29	6·15	3·41	1·13	0·78
		Ep-23	6·75	4·73	7·30	4·38	1·09	0·90
		Ep-31	6·82	4·85	7·65	3·99	1·14	0·81
	Sandy-loam soil	Control	9·52	7·27	10·45	6·20	1·12	0·84
		Ep-23	10·65	8·04	11·81	7·80	1·12	0·96
		Ep-31	10·39	6·89	12·22	6·02	1·12	0·87
$P < F$	Time × O_3		0·419		0·647		0·802	
	SUM00-linear		0·161		0·512		0·837	
	Time × substrate		0·000		0·000		0·145	
	Time × O_3 × substrate		0·914		0·920		0·895	

* Analysis of based on chamber n = 2/ozone level, and a total of 48 plants for each harvest date.

† SUM00 refers to contrasts based on exposure indices given in Table 1.

MANOVA results of data treated as repeated measures (means and probability > F values) for each harvest date.

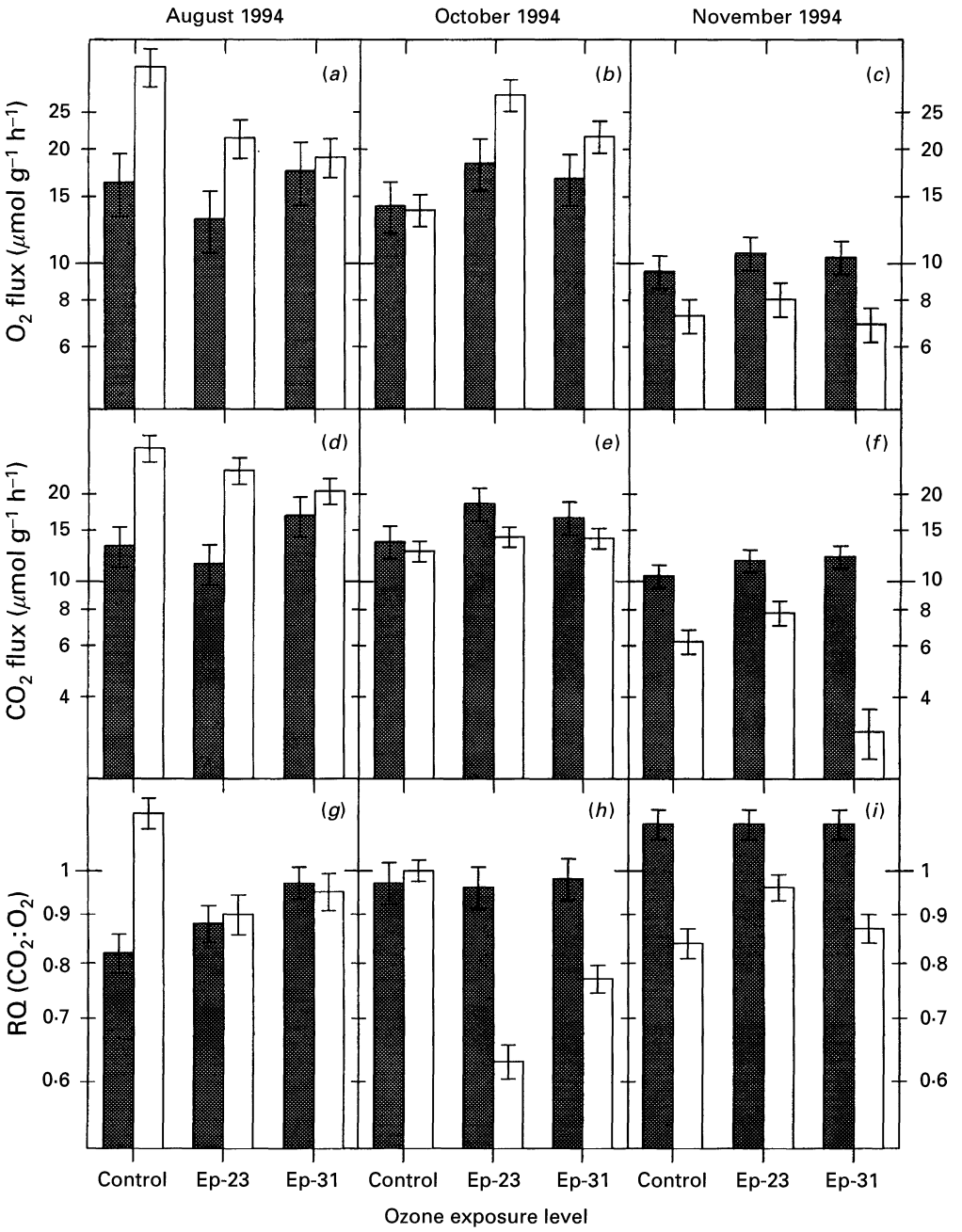

Figure 6. Comparison of August–November 1994, pre-harvest vs. post-harvest O_2 consumption (*a–c*), CO_2 production (*d–f*) and RQ (*g–i*) of soil surrounding roots of ponderosa pine grown in sandy-loam soil and exposed to either Ep-31 O_3, Ep-23 O_3 or charcoal-filtered air (CF). ▨, sandy-loam soil pre-harvest; □, sandy-loam soil post-harvest. Geometric LS means and SE plotted on a $\log_{10}$ *y*-axis (chamber $n = 2$/ozone level for each date; 48 plants in total for each harvest date).

total root system. They assumed that their short-duration measurements of fine-root respiration primarily reflected maintenance respiration, but growth respiration could be a significant proportion of the total. They also found no effect of fertilization on respiration rates of these roots. The inclusion of a wide variety of root sizes and types in our measurements might significantly influence the magnitude of our measured root respiration rates.

Changes in root respiration rates might be related to the shoot ambient conditions. Our respiration measurements were taken under artificial lighting, over a diurnal cycle (unpublished); and the values reported here represent midday respiration rates. Root-respiration rates are generally higher when the shoots are photosynthesizing (Szaniawski & Adams, 1974; Szaniawski, 1980). Increased availability of photosynthate for root respiration resulting from increased production and/or increased translocation has been emphasized as the major factor by which the shoot system controls the root. Diurnal patterns in root respiration can also be explained by fluctua-

tions in the levels of carbohydrate (Farrar, 1981) and the rate of ion uptake (Hansen, 1980) by the root. Szaniawski (1980) found rates of root respiration of Scots pine (*Pinus sylvestris*) seedlings growing in liquid culture was about 15% higher during the day than at night (measured respiration rates ranged from 66 to 130 μmol CO_2 g^{-1} h^{-1}). It is possible that their values were higher than those in the present study because their root-respiration values were calculated only on young, very active tissues with relatively high specific-growth rates.

Our findings suggest that O_3 exposure of young trees can significantly increase soil CO_2 production at certain times of year (Fig. 5*b*, *e*), possibly leading to an annual increase in net CO_2 loss from the below-ground ecosystem. Efflux of CO_2 from the forest floor represents a sizable export of C from terrestrial forest ecosystems (Reichle *et al.*, 1973) and must be accounted for when estimating the ability of forests to sequester C from the atmosphere for long-term storage (Schlesinger, 1990). Ozone exposure of the above-ground portion of a tree might have a significant impact on the magnitude and seasonality of CO_2 flux from soil, potentially changing a significant factor in the C cycling within an ecosystem.

The results from our study suggest that not only does O_3 exposure of Ponderosa pine significantly increase both soil CO_2 production and O_2 consumption, but it also significantly changes the relationship between these respiratory fluxes (RQ), indicating possible disruption of root metabolic activity. The relationship between respiratory O_2 uptake and CO_2 release (RQ) can be used to follow physiological shifts resulting from changes in environmental conditions and root metabolism. Respiratory quotients are thought to reflect changes in substrate utilization, level of non-autotrophic C refixation, and changes in microbial populations and activities. Values of RQ of plant tissue close to unity represent respiration of simple substrates, whereas those below unity represent respiration of more highly reduced substrates. Low RQs might also be related to increases in non-autotrophic C fixation associated with rapid cell division and growth (Andersen & Scagel, 1997). The increase in early season RQ values resulting from O_3 exposure might be a result of lowered carbohydrate reserves in the roots in the spring in O_3-exposed plants (Andersen *et al.*, 1991). Lower RQ values from controls could reflect non-autotrophic C refixation due to rapid root growth and amino acid synthesis.

We found that O_3 exposure increased active fungal and bacterial biomass of the soil surrounding the roots of the trees. Carbon dioxide evolution from soil originates mainly from respiration by living roots of plants and soil microbial activities. Andersen & Rygiewicz (1995) estimated that up to 22% of C fixed by photosynthesis was respired below ground in mycorrhizal plants and 16% in non-mycorrhizal plants. Markkola *et al.* (1995) found that although mycorrhizal fungal biomass decreased along a pollution gradient in northern Finland, biomass of saprophytic fungi increased. Using a combination of treatments such as soil sterilization, treeless blanks, and seedling removals, we have preliminary evidence that suggests the majority of measured CO_2 and O_2 flux in our ponderosa pine system can be attributed to root respiration (unpublished). However, increased active fungal and bacterial biomass in O_3 treatments might result in an increase in the relative proportion of microbial to root respiration compared with that in controls.

Changes in root metabolism following seedling detopping varied with time of year, possibly owing to phenological differences among treatments at the time of detopping. In this experiment, seedling detopping had less of an effect on O_3-exposed plants than on controls in August (Fig. 6); however, below-ground changes following detopping were greater in O_3-exposed plants than in controls in October and November. Plant detopping eliminates C allocation to roots, inhibits protein synthesis and has been used to estimate maintenance and basal respiration rates in roots (Marshall & Perry, 1987). One possible explanation of the seasonal pattern observed in our studies is that in August, root growth and activity were lower in O_3-exposed plants than in CF plants, resulting in a greater effect on root metabolism in controls. Later in the year (October, November), a similar pattern is described in Andersen & Scagel (1997).

In determining the effects of environmental stress on the below-ground respiratory responses of plants, it is useful to differentiate between respiration associated with synthesis of new tissue (growth respiration) from that associated with the maintenance of older tissue (maintenance respiration). In our study we found that O_3 exposure of the shoot altered the patterns of root respiration following detopping. The influence of O_3 exposure on these patterns varied with time of year (Fig. 6). Marshall & Perry (1987) found maintenance respiration of roots of Douglas fir (*Pseudotsuga menzesii*) and ponderosa pine could be estimated by measuring CO_2 efflux from roots 48 h after shoot excision. Using the same technique, they also found that after several days a 'basal' respiration rate was reached, characteristic of non-photosynthetic seedlings depleting their reserves. Although the respiration rates we obtained after detopping of the trees cannot be considered to be actual levels of maintenance respiration, they might reflect changes in protein synthesis and respiratory substrate usage brought about by detopping.

The potential flux of C below ground represents a considerable cost to trees (Dickson, 1989), and below-ground production is estimated to account for

more than 50% of total production in forest ecosystems (Persson, 1979; Keyes & Grier, 1981; Fogel, 1990; Hendrick & Pregitzer, 1993). Several studies have reported decreases in root biomass associated with above-ground exposure to O_3 with concomitant decreases in root:shoot ratios (Cooley & Manning, 1987; Miller, 1987). These studies, along with C-partitioning studies (mostly on annual species) suggest that the proportion of C allocated to the root system is decreased in O_3-exposed plants (McCool & Menge, 1983; McLaughlin & McConathy, 1983; Gorrisen *et al.*, 1991*a*). The mechanisms regulating assimilate partitioning in the whole plant are poorly understood (Wardlaw, 1990; Gifford *et al.*, 1984), and although it is recognized that source-sink relationships change with developmental stage (Evans, 1991), assimilate partitioning is also influenced by both endogenous and environmental factors (Patrick, 1988). The effects of O_3 on CO_2 assimilation vary according to duration of exposure, developmental stage and impact of other stresses (Clark *et al.*, 1995). In our study, we found that O_3 exposure decreased total root d. wt without concomitant decreases in root:shoot ratio (Table 2). Nouchi *et al.* (1991, 1995) describe how Rice (*Oryza sativa*) plants exposed to O_3 exhibited a low root:shoot ratio at the beginning of exposure, but by the end of the season the ratio became close to the control. Considering that we also observed seasonal increases in respiration associated with decreased root d. wt (Figs 2, 3), the proportion of C allocated to the root system might actually be higher in O_3-exposed plants during certain times of the year, resulting in balanced root:shoot ratios at all O_3 levels. This possible scenario is similar to that described by Nouchi *et al.* (1995) with rice. Most C-partitioning studies of trees exposed to O_3 have been short-term at one stage of development; as a result, seasonal and developmental shifts in allocation patterns might not have been detected. Coleman *et al.* (1995) found that mature aspen (*Populus tremuloides*) leaves translocated significantly less C to the roots when exposed to O_3, whereas recently mature leaves increased C allocation to roots with O_3 exposure in an apparently compensatory response. Increased C allocation from recently mature source leaves to roots of O_3-treated plants was at the expense of C allocation to above-ground plant tissues. If O_3 exposure of trees results in seasonal increases in below-ground C allocation and partitioning of substrates to respiratory pools, the below-ground system might be even more of a carbon sink in O_3-exposed forests than previously considered.

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